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6. Project Title: Biofouling by Gregarious Macrofauna: A Multidisciplinary Approach Toward Understanding and Eliminating Founding Individuals.

7. FY94 Publications:

P - Toonen, R.J and J.R. Pawlik. 1994. Foundations of gregariousness. *Nature*, 370, 511-512.

8. References:

P - Pawlik, J.R., C.A. Butman and V.R. Starczak. 1991. Hydrodynamic facilitation of gregarious settlement of a reef-building tube worm. *Science*, 251, 421-424.

P - Toonen, R.J. 1992. Pattern and process: differential growth in aggregations of the gregarious tube worm, *Hydroides dianthus*. Proc. Amer. Acad. Underwater Sciences, 12, 203-213.

P - Pawlik, J.R. and D.J. Mense. 1993. Larval transport, food limitation, ontogenetic plasticity and the recruitment of sabellariid polychaetes. Pages 455-475 in: *Reproduction and Development of Marine Invertebrates*. Edited by Wilson, Jr., W.H., Stricker, S.A. and Shinn, G.L. Johns Hopkins University Press, Baltimore, Maryland.

PS - Pawlik, J.R. and C.A. Butman. 1993. Settlement of a marine tube worm as a function of current velocity: interacting effects of hydrodynamics and behavior. *Limnology and Oceanography*, in press.

PI - Toonen, R.J. and J.R. Pawlik. 1995. Settlement of the gregarious tube worm *Hydroides dianthus* (Serpulidae: Polychaeta): I. All larvae are not created equal. In preparation.

PI - Toonen, R.J. and J.R. Pawlik. 1995. Settlement of the gregarious tube worm *Hydroides dianthus* (Serpulidae: Polychaeta): II. The production of larvae that found aggregations. In preparation.

PI - Toonen, R.J. and J.R. Pawlik. 1995. Settlement of the gregarious tube worm *Hydroides dianthus* (Serpulidae: Polychaeta): III. Cues for gregarious settlement.

IC - Pawlik, J.R. 1992: Chemistry, physics, and behavior: the settlement of marine invertebrate larvae. Invited presentation. 1992 Gordon Research Conference on Marine Natural Products Chemistry; Ventura, California

IC - Pawlik, J.R. and D. Mense. 1992: Chemical and physical factors influencing the settlement of sabellariid polychaetes. Symposium on the Reproduction and Development of Marine Invertebrates; Friday Harbor, Washington.

C - Pawlik, J.R. and C.A. Butman. 1992: Interacting effects of hydrodynamics and behavior on tube worm settlement. 20th Annual Marine Benthic Ecology Meeting; Newport, Rhode Island.

C - Garland, E.D., Butman, C.A. and J.R. Pawlik. 1993. Larval settlement of the polychaete *Hydroides dianthus* in manipulated boundary-layer flow. *First Annual Larval Ecology Meeting*; State University of New York, Stony Brook, NY.

C - Toonen, R.J., Pawlik, J.R. and C.A. Butman. 1993. Founders of aggregations of the tube worm *Hydroides dianthus* are not desperate larvae. *American Society of Limnology and Oceanography*; Edmonton, Canada.

C - Toonen, R.J. and J.R. Pawlik. 1993. For a marine tube worm, all larvae are not created equal. *American Zoologist*, **33**: 118A.

B - Toonen, R.J. 1993. Environmental and heritable components of settlement behavior of *Hydroides dianthus* (Serpulidae: Polychaeta). MS Thesis, University of North Carolina at Wilmington. 82 pp.

9. Statistics:

a) Papers published	4
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c) Books/chapters published	1
d) Books/chapters submitted	0
e) Invited presentations	2
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g) Technical reports	0
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i) Graduate students supported	3
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12. Research Abstract:

**Biofouling by Gregarious Macrofauna:
A Multidisciplinary Approach Toward
Understanding and Eliminating Founding Individuals.**

GOALS

The primary goal of this research is a greater understanding of the physical, chemical and biological processes that control macro-invertebrate biofouling, particularly as it relates to (A) the production of larvae by adults, (B) the supply of larvae to substrata, and (C) the responses of larvae to substata. To realize this goal, we have chosen to examine the processes that control settlement of the common gregarious fouling tube worm, *Hydroides dianthus*.

OBJECTIVES

The proximal objectives of this research are to determine the biological, physical and chemical processes that control the initiation of colony formation of *Hydroides dianthus*. Most fouling invertebrates form aggregations as larvae settle on or near adult conspecifics. While considerable research has focused on larvae that settle gregariously, little is known about the settlement responses of larvae that initially settle on the substratum -- the "founder" larvae that establish aggregations. At UNCW, we are experimentally investigating the effects of substratum characteristics and heredity on the responses of founder and gregariously settling larvae of *H. dianthus*. In a companion study undertaken by Dr. Cheryl Ann Butman and colleagues at Woods Hole Oceanographic Institution, the effects of hydrodynamics on patterns of settlement of *H. dianthus* is concurrently being undertaken.

APPROACH

Laboratory assays of cultured larvae are used to determine whether the production of founder larvae is subject to heredity (i.e., some female worms produce more founder larvae than others), or whether other parameters influence the settlement of founders (egg or larva size, development time, delay of settlement). Assays are also used to determine the substratum requirements for both founder and gregarious settlers, including chemical cues required for the gregarious response.

TASKS COMPLETED

During FY 94 (a period of no-cost extension on this grant, which was due to end 30 Sep 93), we wrote-up our results from the previous years of research, and were successful in getting some of them published in *Nature*. During FY 93, we repeated our experiments from FY 92 to refine our technique and confirm our previous results. We also tested several new hypotheses regarding the importance of nutritional state and the onset of larval maturity and the chemical characteristics of the cue for gregarious settlement. Finally, we compiled our results in the form of a Master's Thesis, and in three publications in preparation. Specifically, the daily settlement over 7 weeks of larvae of *Hydroides dianthus* in response to biofilmed substrata and substrata bearing living adult worms was assessed in sample assays and whole-population assays of batches of larvae. Experimental timecourses were repeated with subpopulations of larvae denied food to assess the importance of nutrition in controlling the response of larvae to substrata. Larvae that settled as founders were raised to adulthood and crossed with other founders for three generations. A North Carolina design sibling-cross experiment with 12 families of worms was performed to assess the heritability of the founder trait. Larval responses to a variety of substratum-associated factors were assayed in still-water experiments; these included surface texture, biofilms and pure microbial cultures, adult tubes and body parts, recently metamorphosed juveniles, and potential spatial competitors. The chemical cue that induces gregarious settlement was further characterized.

RESULTS

Still-water settlement assays of larvae of *Hydroides dianthus* conducted in constant temperature incubators revealed that settlement of founders occurred primarily within the first week of larval competency, whereas substratum specificity of gregarious settlers did not decrease over a planktonic lifespan extended for 70 d of larval culture. Decreased substratum specificity was not induced by starvation; larvae that were denied food reverted to a pre-competent state after 4 d of starvation. Feeding starved cultures after settlement had ceased resulted in larvae becoming competent once again and responding the same way as cultures that were not starved. The majority of both founding and gregarious settlers responded within the first 10 d after fertilization when exposed to suitable substrata daily, but no founders were observed to settle after 14 d. For *Hydroides dianthus*, there appear to be two distinct types of larvae: one that settles indiscriminately on uninhabited substrata (founding settlers), and another that settles only in response to cues associated with conspecific adults (gregarious settlers).

No significant correlations were found between parameters associated with egg production by females and the proportion of founding settlers from a spawn. Artificial selection experiments were ineffective at increasing the proportion of founding settlers produced by the offspring over 3 generations of selection. A North Carolina design sibling cross (NCM1) experiment with 12 families indicated that heritability of founder production is very low (0.0206 ± 0.0104), suggesting that the production of founding settlers does not result from a maternal effect, nor does it appear to be under strong genetic control, although this design cannot be used to assess the importance of paternal inheritance.

For larvae of *H. dianthus* responding to the presence of conspecifics (gregarious settlers), the presence of a single adult is capable of eliciting settlement and juveniles begin to elicit gregarious settlement approximately 96 h after metamorphosis. Settlement was greatest along the anterior 2/5 of

the length of the adult tube. Settlement of larvae in response to conspecific adults, live worms removed from their tubes, and amputated tentacular crowns of live worm were significantly higher than responses to dead worms, empty tubes, or uninhabited biofilmed slides. Larvae settled in response to adults even when prevented from contacting them by a 52-μm mesh, indicating the cue is soluble in seawater and may be perceived at a distance. Extraction of aggregations of adult worms in organic solvents removed the inductive capacity, and this activity was retained in methylene chloride and methanol extracts.

ACCOMPLISHMENTS

We have demonstrated for the first time that a gregariously settling marine invertebrate can produce two distinct larval types: "founding settlers" that settle on any microbially filmed surface soon after the onset of larval maturity, and "gregarious settlers" that respond only to the presence of adult conspecifics and will delay settlement in their absence. This result has important implications in understanding the processes that control recruitment of marine invertebrates and in the design of control measures for the prevention of biofouling. Our major accomplishments were published in a paper that appeared in *Nature* (see attached).

Foundations of gregariousness

SIR — Most benthic marine invertebrates have a pelagic larval phase, during which they may disperse widely. Once ready to metamorphose, the planktonic larvae of many species (for example mussels, oysters, barnacles, sand dollars) settle preferentially on or near conspecific adults, forming monospecific aggregations[1]. Gregarious settlement has many advantages, most notably enhanced adult reproductive success[2], but at the cost of increased intraspecific competition. Aggregations must initially develop from a two-step process: solitary larvae first colonize an uninhabited substratum, then gregarious settlement occurs on or near these 'founders'. To date, research has focused on the latter step[1]. It has been suggested that founders settle because they are unable to locate conspecifics and are incapable of prolonging their planktonic lives[3,4], a concept we term the 'desperate larva' hypothesis. One alternative, however, is that larvae differ in their substratum preferences and that founders are somehow distinct. We have found that females of a tube-dwelling polychaete worm produce larvae that settle in two different ways: one type colonizes uninhabited substrata (founders), whereas the other settles only in response to cues associated with conspecifics (aggregators).

We conducted still-water, single-substratum, laboratory settlement assays with larvae of *Hydroides dianthus*, a common member of epibenthic fouling communities along the east coast of North America. Three experiments were performed, each on a population of 90,000 larvae (pooled from the spawns of about 25 females) cultured in three 4-litre glass jars. We used two types of settlement substrata to assess larval responses: 'biofilmed' slides and 'adult' slides. Biofilmed slides were made by placing etched glass microscope slides into running unfiltered seawater for at least 5 days, so that they became coated with an organic/microbial film. Adult slides were treated similarly, but five small conspecific worms were attached by gluing their tubes to each slide. Two types of settlement assay were conducted until larval cultures were depleted: sample and whole-population assays. For sample assays, a sample of larvae was removed from culture jars and exposed to experimental substrata: for whole-population assays, all remaining larvae were exposed to substrata. Twelve replicate sample assays were run simultaneously in Petri dishes containing a single biofilmed or adult slide and 25 competent larvae. Assay dishes were examined for settlers after 24 h. For whole-population assays, the entire population of larvae was filtered down into a small volume and split evenly among nine replicate glass dishes, each containing a single biofilmed or adult slide. Dishes were placed on a shaker-table rotating at 50 r.p.m. for 1 h, and slides then examined for settlers. Larval types were defined by their responses: founders settled on biofilmed slides, and aggregators settled on adult slides.

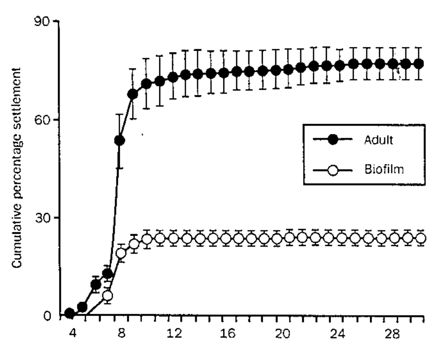

FIG. 1 Mean cumulative percentage settlement ($n = 9$, ± 1 s.e.) of *H. dianthus* when the whole population of larvae was exposed to biofilmed and adult slides daily.

In the first experiment, the whole population was exposed to both biofilmed and adult slides daily after day 3; therefore, all larvae had access to both substrata throughout development. Settlement on both experimental substrata began simultaneously as larvae became competent (Fig. 1), contrary to the prediction of the desperate larvae hypothesis. The bulk of settlement on both biofilmed and adult slides occurred soon after competence, with settlement occurring 7–8 days post-fertilization. Larvae continued to settle on adult slides in low numbers through day 26, but no larvae settled on biofilmed slides after day 14.

In a second experiment, the whole population was exposed to biofilmed slides daily (day 4 through day 14, weekly thereafter), then samples were exposed to adult and biofilmed slides. Under these constraints, only the larvae exposed to adult slides had the opportunity to settle gregariously, whereas the remainder of the population were forced to delay settlement, or settle on biofilmed slides. Surprisingly, virtually all founders settled between 5 and 12 days after fertilization, with the remaining larvae settling only in response to conspecifics (in sample assays) throughout a seven week period (Fig. 2). These results indicate the existence of a distinct subpopulation of larvae that responded to biofilmed substrata; once these individuals were removed, the remaining aggregators delayed settlement in the absence of acceptable conspecific-associated cues. In a similar experiment (not presented here) in which daily exposure of the whole larval population to biofilmed slides was omitted, sample assays revealed the same patterns as seen in Fig. 2 over a period of 70 days, after which, larvae began to die in culture rather than settle in the absence of conspecifics.

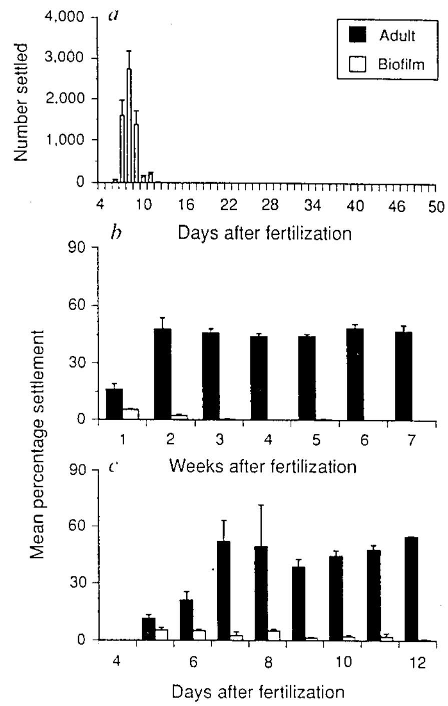

FIG. 2 Settlement of *H. dianthus* when the whole population was exposed to biofilmed slides daily, but only samples were exposed to adult slides. *a*, Number of founding settlers (n = 3, + 1 s.e.) from daily whole-population assays of biofilmed slides. *b*, Weekly mean percentage settlement (n = 12, + 1 s.e.) in sample assays of biofilmed and adult slides. Sample assays were conducted daily for the first 14 days, and weekly thereafter. *c*, Daily mean percentage settlement for the first week, a weekly mean of which is shown in *b*.

The desperate larva hypothesis was advanced on the basis of observations of the development of non-feeding pelagic larvae that exhaust a yolk reserve as they age in the plankton[3,4]. If reduced substratum selectivity is a function of energetic desperation, rather than age alone, it should be possible to test this by starving the larvae of a planktotrophic (feeding) species, such as *H. dianthus*, and monitoring larval responses to substrata. We performed a third experiment, like the second, in which half the population of larvae was starved during days 10–14, and fed and starved subpopulations were

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2. Levitan, D. R. *Am. Nat.* **141**, 517–536 (1993).
3. Knight-Jones, E. W. *J. mar. biol. Ass. U.K.* **32**, 337–345 (1953).
4. Wilson, D. P. *J. mar. biol. Ass. U.K.* **32**, 209–233 (1953).
5. Pawlik J. R. & Mense, D. J. In *Reproduction and Development of Marine Invertebrates* (eds Wilson Jr, W. H. Stricker, S. A. & Shinn, G. L.) (Johns Hopkins Univ. Press, Baltimore, 1994).

assayed separately. We found that settlement of founders could not be induced by starvation. Moreover, starvation resulted in a developmental reversion to a precompetent state, a condition that was reversed when larvae were again provided with food. An ontogenetic shift of this kind was recently described for the larvae of another species of marine polychaete[5].

Besides random settlement, the desperate larva hypothesis is, to our knowledge, the only published explanation for the colonization of new habitats by larvae of gregarious species. The results of our study, a full version of which will be presented in a forthcoming paper, provide an alternative explanation for the process by which monospecific aggregations develop on hard substrata previously uninhabited by conspecifics.

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